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Rolipram inhibits airway microvascular leakage induced by platelet-activating factor, histamine and bradykinin in guinea-pigs

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Abstract—Rolipram (0·1–1000 $\mu g\ kg^{-1}$, i.v.) reduced the increase in microvascular permeability induced by platelet-activating factor (PAF; 50 ng kg^{-1}, i.v.) at different sites of the guinea-pig airways. Rolipram (1–100 $\mu g\ kg^{-1}$, i.v.) inhibited histamine (30 $\mu g\ kg^{-1}$, i.v.)- and bradykinin (0·3 $\mu g\ kg^{-1}$, i.v.)-induced airway microvascular leakage. These effects of rolipram were obtained at doses which inhibit histamine (7–20 $\mu g\ kg^{-1}\ min^{-1}$)-induced bronchoconstriction ($IC50 = 3 \pm 1\ \mu g\ kg^{-1}$, i.v.) without depressing arterial blood pressure in the guinea-pig. Aminophylline (50 mg kg^{-1}) did not change the effect of PAF. The anti-exudative effect of rolipram is of potential therapeutic value in asthma.

Asthma is increasingly recognized as an inflammatory airway disease and increased microvascular permeability is an important component of inflammation. Pharmacological control of vascular leakage may be of interest in asthma because airway oedema contributes not only to airway narrowing but also to bronchial hyper-responsiveness (Boschetto et al 1989; Erjefält & Persson 1991). Despite the relevance of airway plasma exudation in asthma, limited information is available about the influence of anti-asthma drugs on this process (Boschetto et al 1989; Erjefält & Persson 1991).

Theophylline has long been widely used in the management of asthma but its precise mechanism of action remains to be determined. It has been suggested that the partial protection afforded by alkylxanthines against the late-phase response to antigen may be related to an effect on airway vascular permeability (Erjefält & Persson 1991). Evidence has been presented indicating that cyclic nucleotide phosphodiesterase (PDE) inhibition may be significant at therapeutic concentrations of theophylline, and therefore important to its anti-asthma activity (Torphy & Undem 1991). The existence of families of PDE isoenzymes that differ in their tissue distribution is now well recognized (Torphy & Undem 1991). Theophylline is considered a non-selective PDE inhibitor. The synthesis of selective inhibitors for certain PDE isoenzymes has permitted the re-evaluation of the relationship between PDE inhibition and anti-asthma activity. The cyclic GMP-insensitive low K_m, cyclic AMP-specific (type IV) PDE is selectively inhibited by rolipram and other compounds (Reeves et al 1987). The ability to relax airway smooth muscle and the anti-inflammatory activity of selective PDE IV inhibitors make these agents particularly attractive as candidates for asthma therapy (Torphy & Undem 1991).

The purpose of this study was to investigate the effect of rolipram on platelet-activating factor (PAF)-, histamine- and bradykinin-induced microvascular permeability in guinea-pig airways in-vivo. These stimuli were chosen due to their importance as putative inflammatory mediators of asthma. The effect of aminophylline against PAF was also evaluated for comparison.

Materials and methods

Tricoloured (BFA, Panlab, Barcelona, Spain) guinea-pigs, 0·4–0·5 kg, were anaesthetized with urethane (initial dose of 1·5 g kg^{-1}, i.p.; additional doses were given as required to maintain anaesthesia). The carotid artery and jugular vein were cannulated. Catheter patency was assured by flushing with 0·2 mL 0·9% NaCl (saline) containing 100 int. units mL^{-1} heparin. At time 0, vehicle, aminophylline (50 mg kg^{-1} before PAF), or rolipram (0·1, 1, 10, 100 or 1000 $\mu g\ kg^{-1}$ before PAF; 1, 10 or 100 $\mu g\ kg^{-1}$ before histamine or bradykinin) was injected intravenously followed, 9 min later, by Evans blue dye (30 mg kg^{-1}, i.v.). After a further 1 min, PAF (50 ng kg^{-1}), histamine (30 $\mu g\ kg^{-1}$), bradykinin (0·3 $\mu g\ kg^{-1}$) or vehicle (saline containing 0·25% bovine serum albumin for PAF or saline for histamine and bradykinin) was injected intravenously; 5 min later the experiment was terminated. This experimental protocol and the challenge doses were derived, with modifications, from Evans et al (1987), Boschetto et al (1989), Harris et al (1989) and Advenier et al (1992). Doses of drugs refer to base. The injection volume in all interventions was 1 mL kg^{-1}. In experiments with bradykinin, the animals were pretreated with captopril (1 mg kg^{-1}, i.v.)15 min before the beginning of experimentation to inhibit bradykinin metabolism and to enhance its effects. This dose of captopril did not produce significant increase of microvascular leakage in guinea-pig airways (data not shown). In other experiments, the PAF antagonist WEB 2086 (10 $\mu g\ kg^{-1}$, i.v.) was administered 5 min before PAF. At the end of the experiments the intravascular Evans blue was removed in a two-stage process. First, blood was withdrawn from the arterial catheter, while equal amounts of saline were infused through the venous catheter (around 20 mL total). Thereafter, the systemic circulation was perfused with 200 mL saline at a pressure of 120 cm H_2O over 5 min via a large-bore catheter placed into the left ventricle and drained through an incision in the right ventricle. This procedure dilutes venous blood by more than 100-fold (Garland et al 1991). The trachea, main bronchi, and proximal (the proximal 3 mm portion) and distal intrapulmonary airways were excised. Samples of larynx, oesophagus, and bladder were also obtained. Evans blue was extracted by incubating tissues in 2 mL formamide at 37°C for 16 h and its concentration determined by light absorbance at 620 nm (Uvikon 940 spectrophotometer; Kontron). The extracted Evans blue was quantified by interpolation on a standard curve of dye concentration (0·5–10 $\mu g\ mL^{-1}$). Corrections were made for non-Evans blue-related absorbance at 620 nm. Evans blue dye content of each sample was expressed as ng (mg wet wt tissue)$^{-1}$. The Evans blue dye technique has previously been shown to correlate closely with the extravasation of radiolabelled albumin in guinea-pig airways (Rogers et al 1989).

In a separate group of animals, the mean arterial blood pressure was monitored and the responses to rolipram (0·1–1000 $\mu g\ kg^{-1}$, i.v.) recorded. In other experiments, guinea-pigs were anaesthetized and prepared for the recording of airway pressure from the side arm of a tracheal cannula. Histamine was infused (7–20 $\mu g\ kg^{-1}\ min^{-1}$, i.v.) to elevate airway pressure by 15–25 mmHg (150–200% of baseline). Once a steady-state bronchoconstrictor response was observed rolipram or vehicle was administered intravenously in ascending doses (seven doses per animal). Bronchodilatation was expressed as the % inhibition of the histamine-induced bronchoconstriction and the inhibitory concentration 50% (IC50) was calculated from the dose-response relationship by linear regression analysis.

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Drugs were dissolved in saline. The stock solution for rolipram was prepared in dimethylsulphoxide and then diluted in saline, as appropriate. Data are expressed as mean ± s.e.m. Statistical analysis of the results was performed by the one-way analysis of variance or Student's *t*-test (unpaired, two-tailed). When more than one comparison was made, one-way analysis of variance was followed by the Bonferroni corrections. Probability values of $P < 0.05$ were considered significant.

Results

In sham-operated animals (i.e. animals not receiving drug vehicles, drug treatments or extravasation stimuli but otherwise treated in a similar way), basal values for Evans blue dye content of trachea, main bronchi, proximal and distal intrapulmonary airways were respectively 5·9 ± 3·4, 10·2 ± 6·5, 11·6 ± 5·4 and 9·1 ± 3·4 ng mg^{-1} (n = 5). At all airway levels no significant difference in Evans blue dye extravasation was observed among control groups treated with the vehicles (saline, n = 5; bovine serum albumin in saline, n = 5; or dimethylsulphoxide, n = 5). The results were pooled and are presented in Fig. 1. These control values are not significantly different ($P > 0.05$) from basal values in sham-operated animals. The values for Evans blue dye content of trachea, main bronchi, proximal and distal intrapulmonary airways in animals treated with rolipram (1000 μg kg^{-1}) but not receiving extravasation stimuli were, respectively, 9·4 ± 5·1, 15·3 ± 8·9, 17·8 ± 9·3 and 15·3 ± 8·8 ng mg^{-1} (n = 5); these values were not significantly different ($P > 0.05$) from basal values.

PAF (50 ng kg^{-1}) increased microvascular permeability in all tissues examined (Fig. 1). Pretreatment with WEB 2086 (10 μg kg^{-1}) inhibited the effects of PAF. Thus, Evans blue dye content of trachea, main bronchi, proximal and distal intrapulmonary airways in the animals treated with WEB 2086 was respectively 22·4 ± 3·0, 28·3 ± 6·1, 28·6 ± 5·7, 21·2 ± 2·6 ng mg^{-1} (n = 6; $P < 0.05$ compared with the corresponding control PAF values). Comparison of these data with those obtained in animals receiving PAF but not treated with WEB 2086 show that the WEB 2086-induced reductions in the response to PAF were 60·7, 66·3, 63·3 and 66·6%, respectively. Aminophylline (50 mg kg^{-1}) failed to decrease the effect of PAF (Evans blue dye content of trachea, main bronchi, proximal and distal intrapulmonary airways was 47·1 ± 3·7, 65·5 ± 9·3, 69·5 ± 6·9, 45·9 ± 3·6 ng mg^{-1}, respectively; n = 4; not significantly different from control PAF). In contrast, rolipram (0·1–1000 μg kg^{-1}) reduced the increase in microvascular permeability induced by PAF at different sites of the airways. Complete inhibition of the effect of PAF in all airway levels was only achieved with the highest dose (1000 μg kg^{-1}) of rolipram tested but lower doses (10 or 100 μg kg^{-1}) produced total inhibition in bronchi and intrapulmonary airways (Fig. 1).

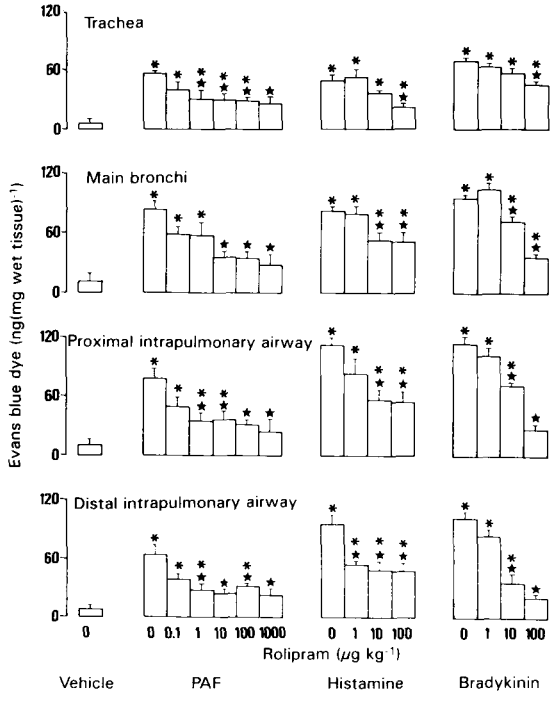

FIG 1. Histograms showing the inhibition by rolipram of PAF (50 ng kg^{-1})-, histamine (30 μg kg^{-1})-, and bradykinin (0·3 μg)-induced microvascular leakage in guinea-pig airways. Means (s.e.m. shown by vertical bars) of 5–15 animals are shown. * $P < 0.05$ compared with vehicle values; * $P < 0.05$ compared with values from corresponding control.

Histamine (30 μg kg^{-1}) caused a significant increase in airways microvascular leakage comparable to PAF (50 ng kg^{-1}; Fig. 1). Rolipram (1 μg kg^{-1}) reduced histamine-induced leakage at distal intrapulmonary airways. Higher doses of rolipram (10–100 μg kg^{-1}) extended this anti-exudative effect to proximal airways (Fig. 1) but failed to block completely the effects of histamine. Bradykinin (0·3 μg kg^{-1}) enhanced microvascular leakage in guinea-pig airways (Fig. 1); this effect was significantly reduced by rolipram (10 μg kg^{-1}) at different sites of the respiratory tract and fully inhibited by rolipram (100 μg kg^{-1}) in the intrapulmonary airways. The effects of rolipram against PAF-, histamine- and bradykinin-induced vascular leakage showed a tendency to be dose-related (Fig. 1) but differences between adjacent doses failed to reach statistical significance in most cases. Inhibitory effects of rolipram were also exerted on PAF-, histamine- and bradkykinin-induced vascular leakage in larynx, oesophagus and bladder (data not shown).

Baseline mean arterial blood pressure was 56 ± 3 mmHg (n = 6). Rolipram (0·1–1000 μg kg^{-1}, i.v.) produced small and transient decreases of arterial blood pressure. After 15 min of the administration of rolipram, the mean arterial blood pressure was not significantly different from pre-drug values except for the highest dose tested which produced a sustained reduction of pressure (7 ± 2%; n = 6; $P < 0.05$ from baseline). Rolipram inhibited in a dose-dependent manner the bronchoconstriction elicited by histamine. A dose of 0·1 μg kg^{-1} produced 9 ± 7% inhibition, and 100 μg kg^{-1} was a near-maximal dose (80 ± 7% inhibition). The IC50 was 3·0 ± 1·0 μg kg^{-1} (n = 6).

Discussion

The main finding of the present study is that rolipram, a selective inhibitor of PDE IV (Reeves et al 1987), inhibits airway microvascular leakage induced by intravenous PAF, histamine or bradykinin in guinea-pigs. The vascular leakage produced by the stimuli used in the present study was not restricted to the tracheobronchial circulation since leakage was also observed in oesophagus and bladder. This is a limitation of the technique due to the intravenous administration of the stimuli. The anti-exudative effect of rolipram on leakage in other tissues is beyond the scope of the present work and has not been analysed.

It has recently been shown that rolipram (200 μg, intratracheally, or 25 mg kg^{-1}, p.o.) inhibits PAF (4 nM intratracheally)-induced microvascular leakage in guinea-pig airways (Raeburn et al 1991; Raeburn & Karlsson 1992). These present results therefore are in good agreement with those previously reported. The effects of PAF or histamine producing plasma exudation in

the guinea-pig airways is considered a direct effect (Evans et al 1987) and PAF- or histamine H_1-receptor antagonists block these effects (Evans et al 1987; this study). However, the participation of mediators (products of cyclo-oxygenase or lipoxygenase pathways) in the effect of these inflammatory stimuli cannot be completely excluded (Evans et al 1987). Similarly, the effect of bradykinin on microvascular permeability in the airways is a direct effect, blocked by selective antagonists of bradykinin BK_2 receptors (Ichinose & Barnes 1990) but also involves the release of a number of inflammatory mediators. Selective inhibitors of PDE IV reduce the release of mediators from inflammatory cells (Dent et al 1991) and may also act to decrease airway eosinophilia and inflammation (Torphy & Undem 1991).

The anti-leakage effect of rolipram in the airways is probably unrelated to changes in mucosal blood flow since selective inhibitors of PDE IV do not have marked vasodilator potential (Heaslip et al 1991) and vasodilatation would augment rather than diminish microvascular permeability. The anti-exudative effects of rolipram were obtained with doses in the range of those required in-vivo for inhibition of histamine-induced bronchoconstriction (Harris et al 1989; Heaslip et al 1991; this study). However, in the concentration range of 10–100 $\mu g\ kg^{-1}$, rolipram reduced but did not completely inhibit the effects of histamine. The partial inhibition produced by rolipram is not attributable to the use of a supramaximal stimulus since the effect of histamine (30 $\mu g\ kg^{-1}$) is submaximal (Evans et al 1987). In fact, rolipram, in the same dose range, abolished the effect of submaximal stimuli with PAF (50 $ng\ kg^{-1}$) or bradykinin (0·3 $\mu g\ kg^{-1}$) which produce approximately the same amount of leak at different sites of the airways (this study). The partial inhibition of the histamine-evoked plasma exudation may represent a residual effect of histamine which is not susceptible to rolipram. Similarly, Advenier et al (1992) found that β_2-adrenoceptor agonists inhibit bradykinin completely and histamine partially but the precise explanation for these findings is not known.

The effects obtained with rolipram probably represent a case for functional antagonism of stimuli. The mechanisms of leakage into the airways have not been elucidated. Extravasation involves endothelial cells in postcapillary venules of subepithelial microvessels. The presence of PDE IV in endothelial cells of large arteries has been demonstrated (Lugnier & Schini 1990). This permits speculations about PDE IV being a modulator of microvascular leakage in the tracheobronchial circulation.

In contrast with the results obtained with rolipram, the non-selective PDE inhibitor theophylline, administered as aminophylline (50 $mg\ kg^{-1}$, i.v.), failed to inhibit PAF-induced airway microvascular leakage. This dose gives theophylline plasma levels around 40 $mg\ L^{-1}$ in the guinea-pig (Boschetto et al 1989) i.e. well above therapeutic plasma levels found in man. The lack of anti-exudative effects of theophylline in the guinea-pig airways is in accordance to the results obtained by Boschetto et al (1989) for aminophylline (12·5–50 $mg\ kg^{-1}$, i.v.) with an experimental technique and protocol similar to those used in the present study. Raeburn et al (1991) found that theophylline (25 $mg\ kg^{-1}$, p.o.) failed to inhibit PAF (4 nM, intratracheally)-induced plasma extravasation into guinea-pig airways. However, results from the same laboratory (Raeburn & Karlsson 1992) showed that theophylline (200 μg, intratracheally) partly inhibited PAF (4 nM, intratracheally)-induced vascular permeability in guinea-pig bronchi but was without effect in the trachea. Theophylline has also been reported to inhibit the leakage of plasma proteins into the guinea-pig airways induced by intratracheally administered PAF (Persson et al 1987) or other inflammatory stimuli (Erjefält & Persson 1991). Differences in the route of administration (directly into airways vs systemic) and differences in the techniques and protocols among laboratories may, at least in part, account for these contradictory findings.

In conclusion, our results show that rolipram, a selective inhibitor of PDE IV, inhibits PAF-, histamine- and bradykinin-induced microvascular leakage in the guinea-pig airways. This effect was not observed for theophylline. The anti-exudative effect of rolipram may be of potential therapeutic value in the management of asthma.

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